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Analytical Method Development and Validation for the Simultaneous Estimation of Tamsulosin and Dutasteride in Its Combined Tablet Dosage Form by UV Spectrophotometry and RP-HPLC Methods

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Abstract: ***Objective: The proposed method was new precise, simple, fast, accurate method for the analytical method development and validation for the simultaneous estimation of Tamsulosin and Dutasteride in its combined tablet dosage form by UV spectrophotometry and RP HPLC methods. Determine the absorption maxima of both the drugs in UV-Visible region in different solvents/ buffers and selecting the solvents for HPLC method development.***

Method: A simple and selective HPLC method was described for the determination of Tamsulosin hydrochloride and Dutasteride tablet dosage forms. Chromatographic separation was achieved on a C_{18} column using mobile phase consisting of a mixture of Ammonium acetate Buffer pH: 3.5: Acetonitrile: Methanol (40:30:30v/v), with detection of 223 nm.

Result: Linearity was observed in the range 6.4-44.8 µg /ml for Tamsulosin hydrochloride (r^2 =0.999) and 8-56µg /ml for Dutasteride (r^2 =0.9961) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The accuracy of the method was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. The validation of method was carried out utilizing ICH-guidelines.

Conclusion: From the experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Tamsulosin hydrochloride and Dutasteride was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis.

Keywords: Tamsulosin, Dutasteride, RP-HPLC, Methanol, Ammonium acetate buffer pH 3.5 , C18 column and 223nm .

1. Introduction

Tamsulosin is a selective antagonist at alpha-1A and alpha-1B-adrenoceptors in the prostate, prostatic capsule, prostatic urethra, and bladder neck. At least three discrete alpha1-adrenoceptor subtypes have been identified: alpha-1A, alpha-1B and alpha-1D; their distribution differs between human organs and tissue. Approximately 70% of the alpha1-receptors in human prostate are of the alpha-1A subtype. Blockage of these receptors causes relaxation of smooth muscles in the bladder neck and prostate.[1,2] Dutasteride belongs to a class of drugs called 5-alpha-reductase inhibitors, which block the action of the 5-alpha-reductase enzymes that convert testosterone into dihydrotestosterone (DHT).[3] The combination of tamsulosin and dutasteride is used to treat benign prostatic hyperplasia (BPH) in men with an enlarged prostate.[4] A new method is developed for the simultaneous estimation and validation of tamsulosin and dutasteride .

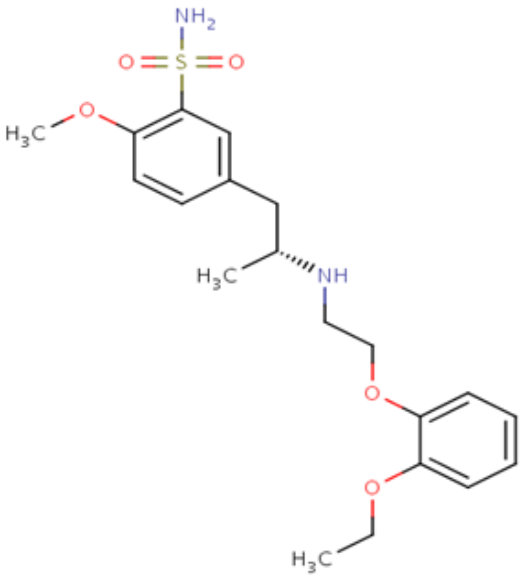

Fig.no.1 Structure of Tamsulosin [5]

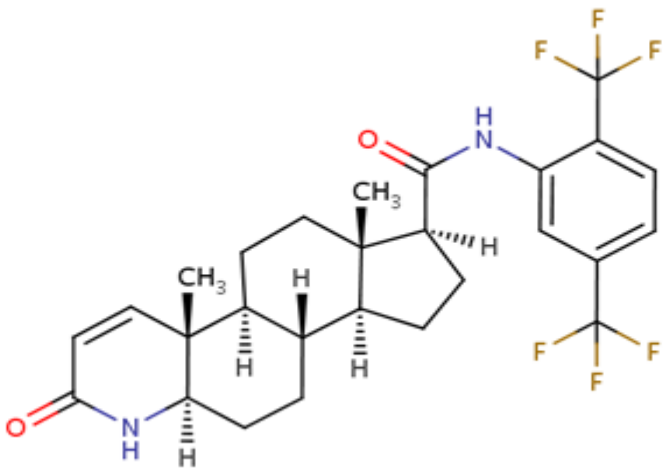

Fig.no.2 Structure of Dutasteride [6]

2. MATERIALS AND METHOD

Reagents and Materials

Water: HPLC grade, Sodium dihydrogen ortho phosphate: AR grade, Methanol: HPLC grade, Potassium Dihydrogen ortho Phosphate: AR grade, Acetonitrile :HPLC grade , Ammonium acetate :AR grade, tetra hydro furan: AR grade.

Drugs used

Tamsulosin hydrochloride and Dutasteride bulk drugs gift samples were obtained from Chandra labs , Hyd , Veltam plus (0.4+0.5) (Tamsulosin 0.4 mg and Dutasteride 0.5 mg label claims) manufactured by: Arron (Intas Pharmaceuticals Ltd.), India which was obtained from local pharmacy.

Instruments used

UV-Visible Spectrophotometer Nicolet evolution 100, HPLC Shimadzu(LC 20 AT VP) , HPLC Agilent 1200 series, Ultra sonicator Citizen, Digital Ultrasonic Cleaner , pH meter Global digital , Electronic balance Shimadzu, Syringe Hamilton ,HPLC column Kromosil C18 column (250×4.6mm× 5µ) .

Preparation of mobile Phase

A mixture of 40 volumes of 20mM Ammonium acetate buffer pH 3.5:30 volumes of Acetonitrile: 30 volumes of Methanol. The mobile phase was sonicated for 10min to remove gases.

Preparation of Ammonium acetate buffer (20mM):

0.15416 gm. of Ammonium acetate was weighed and dissolved in 100ml of water and volume was made up to 100ml with water, pH to 3.5 using orthophosphoric acid. The buffer was filtered through 0.45µ filters to remove all fine particles and gases. [7]

3. METHOD

Weigh accurately 40 mg of Tamsulosin hydrochloride and 32 mg of Dutasteride in 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase. From above stock solution 40 µg/ml of Tamsulosin hydrochloride and 32 µg/ml of Dutasteride is prepared by diluting 5ml to 50ml with mobile phase. This solution is used for recording chromatogram. And the chromatographic conditions are Mobile phase as ammonium acetate buffer: acetonitrile: methanol in the ratios 40:30:30 and Kromosil C18 column (250×4.6mm× 5µ) and wave length at 223 nm, flow rate 1.2ml/min and the buffer pH 3.5. And the chromatogram was shown in the fig below and the efficiency and the retention time are satisfactory.

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting UV-Visible spectrum of **Tamsulosin hydrochloride** and **Dutasteride** and the isosbestic point was 223 nm shown below.

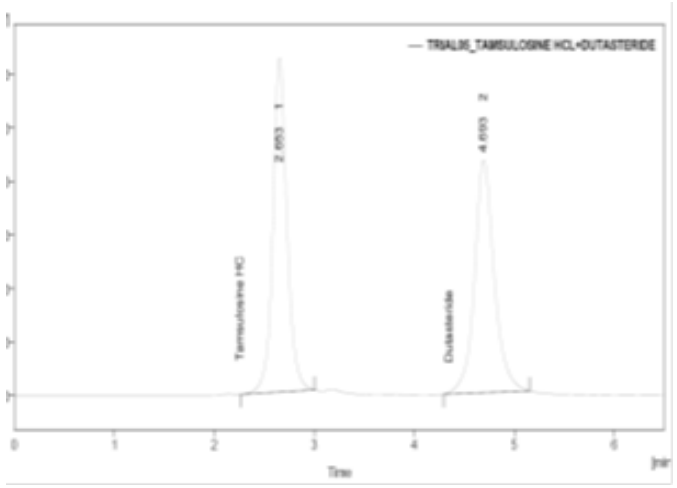

Fig.no.3: Chromatogram of Tamsulosin hydrochloride and Dutasteride by using mobile phase

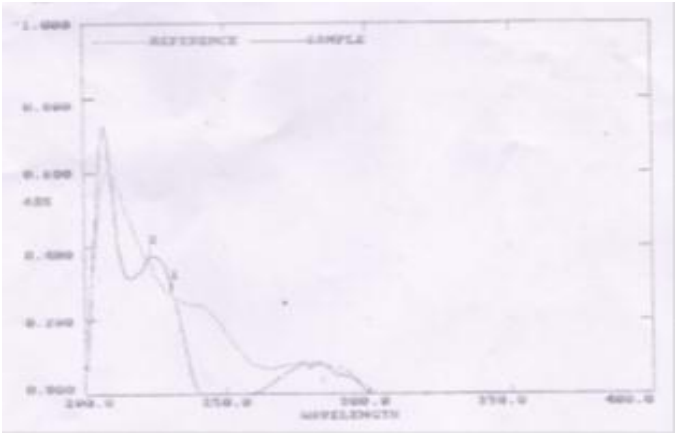

Fig.no.4: UV-VIS spectrum of Tamsulosin hydrochloride and Dutasteride and the isosbestic point was 223 nm.

Assay

Preparation of Standard sample

Standard stock solutions of Tamsulosin hydrochloride and Dutasteride (microgram/ml) were prepared by dissolving 40 mg of Tamsulosin hydrochloride and 32 mg of Dutasteride dissolved in sufficient mobile phase. After that the solution using 0.45-micron syringe filter and sonicated for 5min and then to100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml of Tamsulosin hydrochloride and 32µg/ml of Dutasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Preparation of Tablet sample

10tablets (each tablet contains 0.5 mg of Tamsulosin hydrochloride and 0.4 mg of Dutasteride) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Tamsulosin hydrochloride (40µg/ml) and Dutasteride

(32µg/ml) were prepared by dissolving weight equivalent to 40 mg of Tamsulosin hydrochloride and 32 mg of Dutasteride and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml of Tamsulosin hydrochloride and 32µg/ml of Dutasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase. [8]

Calculation

The amount of Tamsulosin hydrochloride and Dutasteride present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Where,

AS: Average peak area due to standard preparation, AT: Peak area due to assay preparation

WS: Weight of Tamsulosin hydrochloride and Dutasteride in mg, WT: Weight of sample in assay preparation, DT: Dilution of assay preparation. It is observed that the amount of Tamsulosin hydrochloride and Dutasteride present in the taken dosage form was found to be 98.93 % and 99.16% respectively.

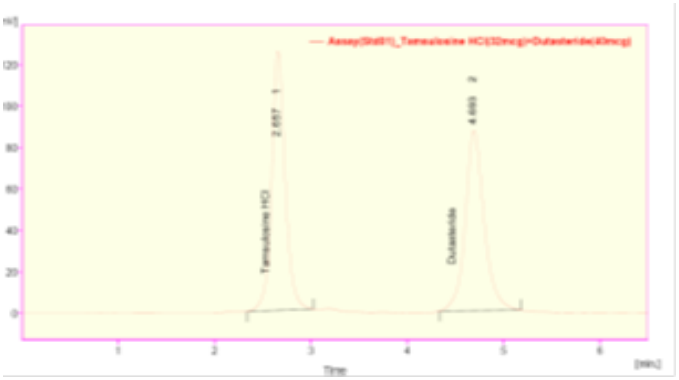

Fig. no.5: Chromatogram of Assay standard preparation-1

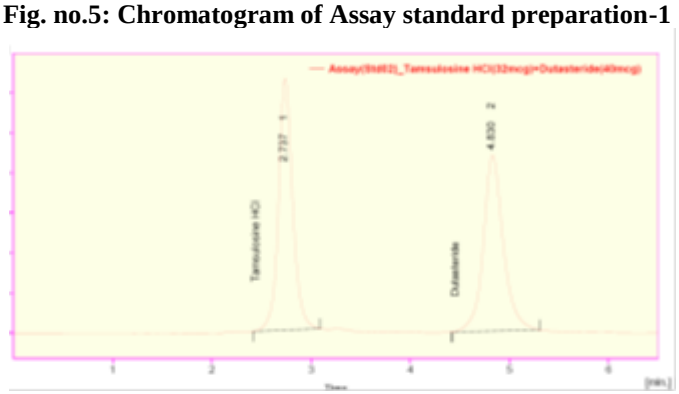

Fig.no.6: Chromatogram of Chromatogram of Assay standard preparation-2

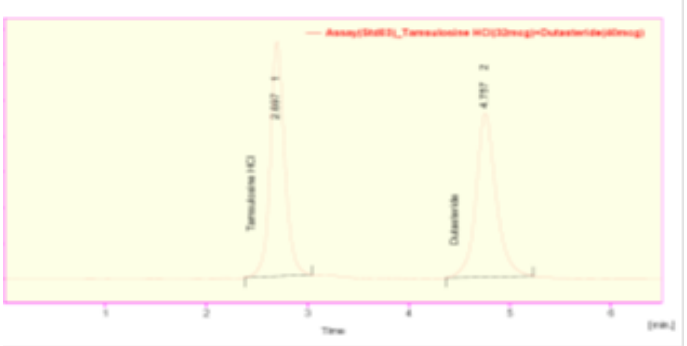

Fig.no. 7: Chromatogram of Assay standard preparation-3

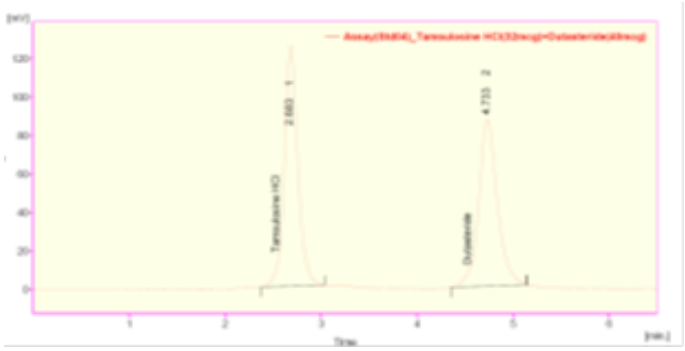

Fig.no.8: Chromatogram of Assay standard preparation-4

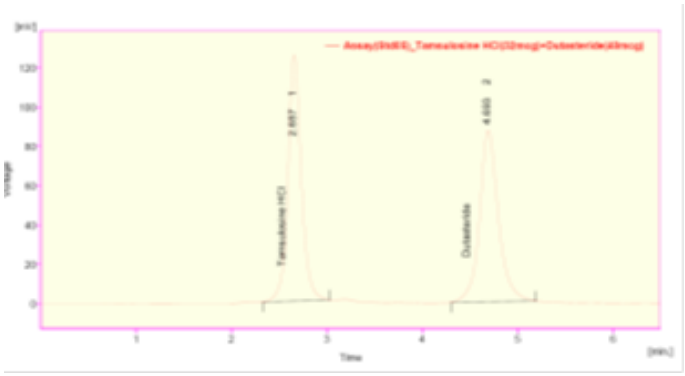

Fig.no.9: Chromatogram of Assay standard preparation-5

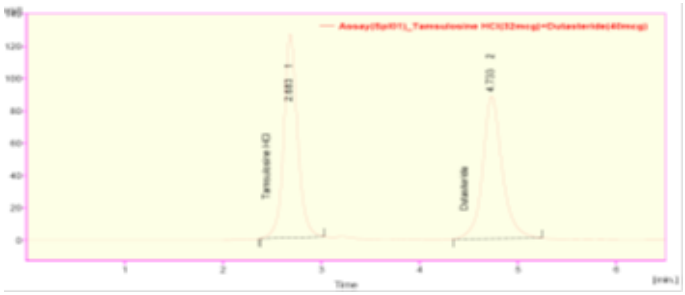

Fig.no.10: Chromatogram of Assay sample preparation-1

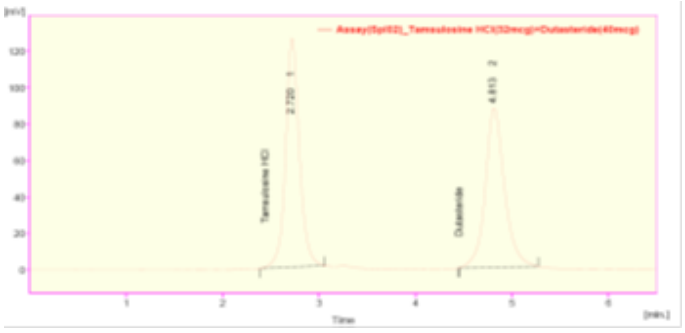

Fig.no.11: Chromatogram of Assay sample preparation-2

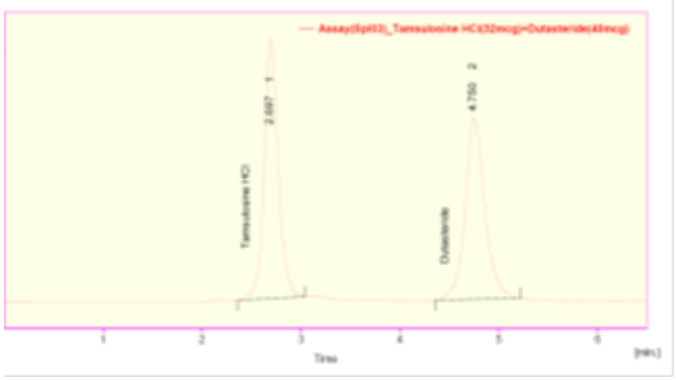

Fig.no.12: Chromatogram of Assay sample preparation-3

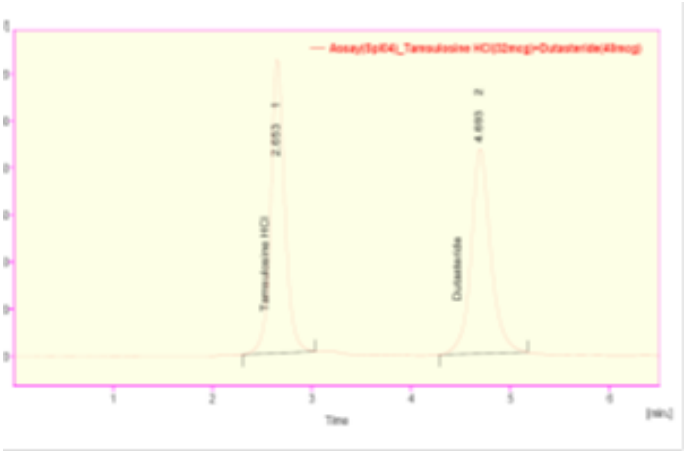

Fig.no.13: Chromatogram of Assay sample preparation-4

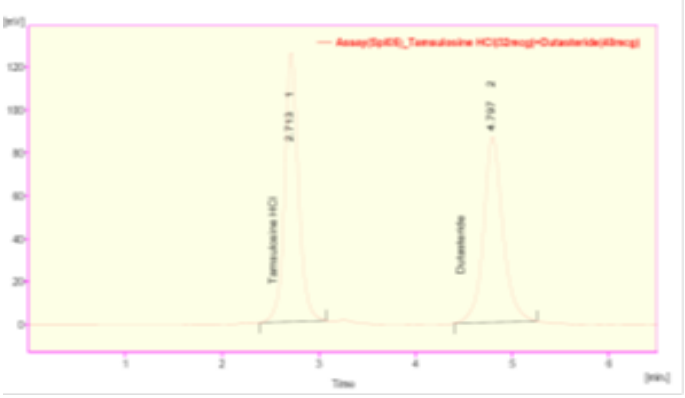

Fig.no.14: Chromatogram of Assay sample preparation-5

Table No.1: **Assay Results**

Tamsulosin hydrochloride			**Dutasteride**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	1218.293	1229.507	1183.851	1216.259
Injection-2	1248.324	1246.813	1228.600	1215.705
Injection-3	1289.749	1236.109	1270.837	1215.781
Injection-4	1222.300	1228.810	1175.298	1196.616
Injection-5	1221.445	1241.937	1198.939	1204.885
Average Area	1240.022	1236.635	1211.505	1209.849
Tablet average weight	3.5		3.5	
Standard weight	32		40	
Sample weight	280		280	
Label amount	0.4		0.5	
std. purity	99.2		99.3	
Amount found in mg	0.40		0.50	
Assay(%purity)	98.93		99.16	

4. METHOD VALIDATION

Method validation is defined as the process of proving that an analytical method is acceptable for its intended use. The following methods are validated as per ICH Guidelines. [9-19]

System suitability

Standard solutions were prepared as per the test method and injected into the chromatographic system. The system suitability parameters like theoretical plates, resolution and asymmetric factor were evaluated.

Table No.2: Results for system suitability of Tamsulosin and Dutasteride

S. No	**Name**	**Rt (min)**	**Peak Area**	**Theoretical plates (TP)**	**Asymmetry**	**Efficiency**	**Resolution**
1	**Tamsulosin**	2.6937	1230.180	2813	**1.192**	**2913**	-
2	**Dutasteride**	4.761	1199.342	3051	**1.231**	**3017**	**6.959**

Specificity by Direct comparison method

There is no interference of mobile phase, solvent and placebo with the analyte peak and also the peak purity of analyte peak which indicate that the method is specific for the analysis of analytes in their dosage form.

Preparation of samples for Assay

Standard sample

Standard stock solutions of Tamsulosin hydrochloride and Dutasteride (microgram/ml) were prepared by dissolving 40 mg of Tamsulosin hydrochloride and 32 mg of Dutasteride dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5min and dilute to 100 ml with mobile phase. Further dilutions are prepared in 5 replicates of 40 µg/ml of Tamsulosin hydrochloride and 32 µg/ml of Dutasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Tablet sample

10 Tablets (each tablet contains 0.5 mg of Tamsulosin hydrochloride and 0.4 mg of Dutasteride) were weighed and taken into a mortar uniformly mixed. Test stock solutions of Tamsulosin hydrochloride (40µg/ml) and Dutasteride (32µg/ml) were prepared by dissolving weight equivalent to 40 mg of Tamsulosin hydrochloride and 32 mg of Dutasteride and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5 min and dilute to 100ml with mobile phase. Further dilutions are prepared in 5 replicates of 40µg/ml of Tamsulosin hydrochloride and 32 µg/ml of Dutasteride was made by adding 1 ml of stock solution to 10 ml of mobile phase.

Fig.no.15: Blank chromatogram for specificity by using mobile phase

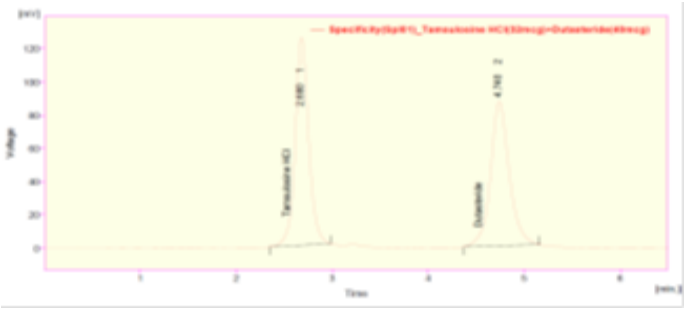

Fig.no.16: Chromatogram for specificity of Tamsulosin hydrochloride and Dutasteride sample (Veltam Plus)

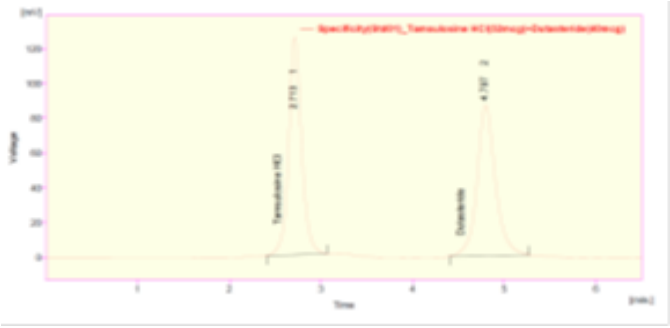

Fig.no.17: Chromatogram for Specificity of Tamsulosin hydrochloride and Dutasteride standard

Linearity and range

Standard stock solutions of Tamsulosin hydrochloride and Dutasteride (microgram/ml) were prepared by dissolving 40 mg of Tamsulosin hydrochloride and 32 mg of Dutasteride dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and sonicated for 5min and dilute to 100 ml with mobile phase and further dilutions were made, seven injections were taken for each drug and noted down the concentrations and areas and linearity graphs were plotted shown in fig18&19 below.

Table.No.3: Recovery results for Tamsulosin hydrochloride

Recovery level	Accuracy Tamsulosin hydrochloride					Average % Recovery
	Amount taken (mcg/ml)	Area	Average area	Amount recovered(mcg/ml)	%Recovery	
80%	32	1245.776	1243.999	32.11	100.34	100.23%
	32	1242.478				
	32	1243.744				
100%	38.4	1585.507	1588.772	37.88	98.65	
	38.4	1592.771				
	38.4	1588.				
120%	44.8	1806.204	1863.448	45.57	101.72	
	44.8	1891.				
	44.8	1892.				

Conc

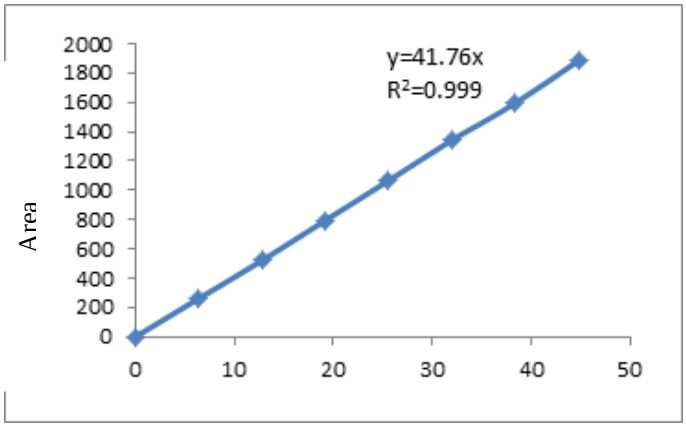

Fig.no.18: Linearity graph of Tamsulosin hydrochloride

Conc

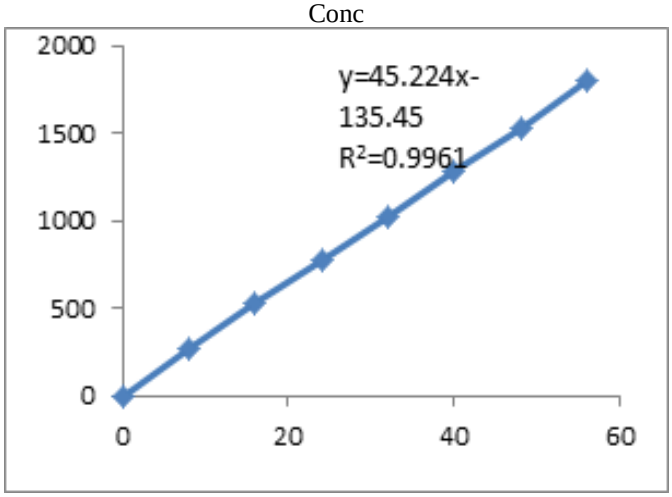

Fig.no .19: Linearity graph of DutasterideAccuracy

To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 80%, 100%, 120%. The percentage recovery and mean recovery are estimated below table 3&4.

Table.No.4: Recovery results for Dutasteride

Recovery level	Accuracy Dutasteride					Average % Recovery
	Amount taken(mcg/ml)	Area	Average area	Amount recovered (mcg/ml)	% Recovery	
80%	40	1225.647	1209.38	39.29	98.23	99.47%
	40	1195.931				
	40	1206.561				
100%	48	1534.413	1529.259	47.65	99.26	
	48	1527.826				
	48	1525.537				
120%	56	1795.856	1802.554	56.52	100.92	
	56	1795.416				
	56	1816.391				

Precision

Prepared sample preparations of Tamsulosin hydrochloride and Dutasteride as per test method and injected 6 times in to the column. The %RSD is estimated in below table.

Table No.5: Precision of Tamsulosin and Dutasteride

Tamsulosin hydrochloride		
S.No.	Rt	Area
1	2.73	1242.86
2	2.713	1235.363
3	2.703	1235.137
4	2.68	1233.439
5	2.683	1208.632
6	2.653	1225.648
Avg	2.6937	1230.18
St dev	0.0273	11.897
%RSD	1.01	0.97

Dutasteride		
S.No.	Rt	Area
1	4.823	1206.845
2	4.797	1197.84
3	4.78	1196.066
4	4.74	1192.851
5	4.733	1196.885
6	4.693	1205.564
Avg	4.761	1199.342
St dev	0.048	5.589
%RSD	1	0.47

Limit of Detection

$$LOD = \frac{3.3\sigma}{S}$$

Where, σ = the standard deviation of the response, S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte. Calibration graphs of Tamsulosin hydrochloride and Dutasteride are shown above fig.no18, 19.

Results for calibration graph was shown in table below.

The LOD for this method was found to be 0.798 µg/ml & area 33.46 for Tamsulosin hydrochloride and 0.922 µg/ml & area 41.79 for Dutasteride.

Table.No.6: Calibration results

S.No	Tamsulosin hydrochloride		Dutasteride	
	Concentration µg/ml	Peak Area	Concentration µg/ml	Peak Area
1	6.4	261.237	8	270.599
2	12.8	520.755	16	525.652
3	19.2	794.273	24	773.801
4	25.6	1066.791	32	1018.95
5	32	1342.066	40	1283.845
6	38.4	1594.287	48	1532.898
7	44.8	1885.204	56	1795.856
S.D.				
Slope	41.76		45.224	

Limit of Quantification

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = the standard deviation of the response, S = the slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

The LOQ for this method was found to be 2.418 µg/ml & area 101.40 for Tamsulosin hydrochloride and 2.796 µg/ml & area 126.64 for Dutasteride.

Robustness

To demonstrate the robustness of the method, prepared solution as per test method and injected at different variable conditions like using different conditions like Temperature and wavelength. System suitability parameters were compared with that of method precision.

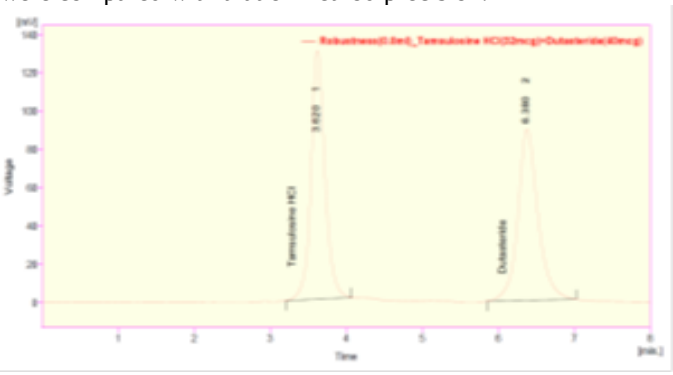

Fig.no.20: Chromatogram of Tamsulosin hydrochloride and Dutasteride Robustness (Flow: 0.8 ml/min)

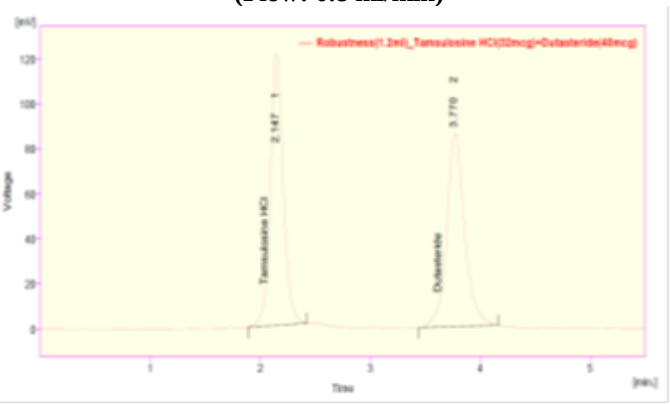

Fig.no.21: Chromatogram of Tamsulosin hydrochloride and Dutasteride Robustness (Flow: 1.2 ml/min)

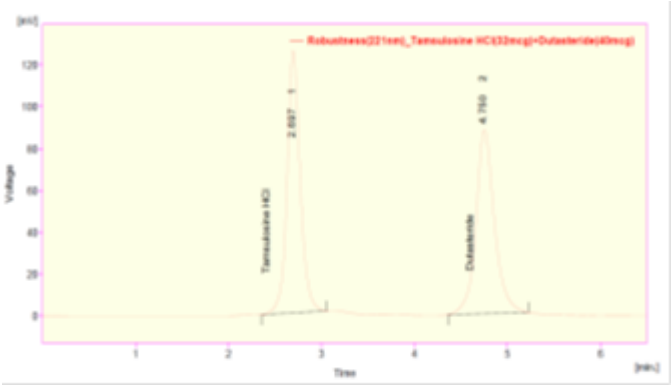

Fig.no.22: Chromatogram of Tamsulosin hydrochloride and Dutasteride for Robustness (221nm)

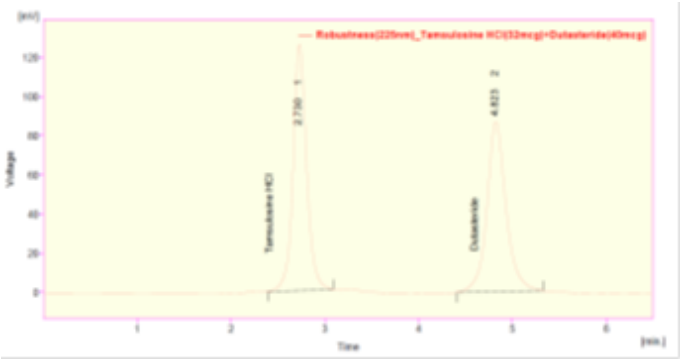

Fig.no.23: Chromatogram of Tamsulosin hydrochloride and Dutasteride for Robustness (225nm)

Table.No.6: Result of Robustness study

Parameter	Tamsulosin hydrochloride		teride	
	Retention time (min)	Tailing factor	Retention time (min)	Tailing factor
Flow **0.8ml/min** **1.0ml/min** **1.2ml/min**				
Wavelength **221nm** **223nm** **225nm**				

Ruggedness

The ruggedness of the method was studied by the determining the analyst to analyst variation by performing the Assay by two different analysts.

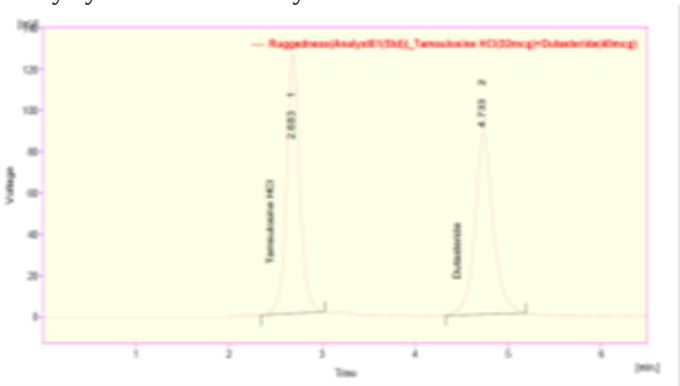

Fig.no.24: Chromatogram of Analyst 01 standard preparation

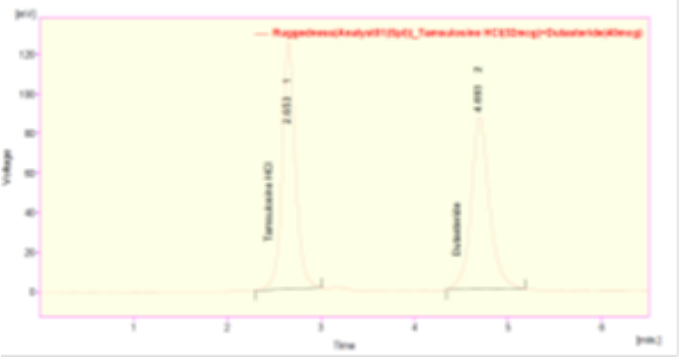

Fig.no.25: Chromatogram of Analyst 01 sample preparation

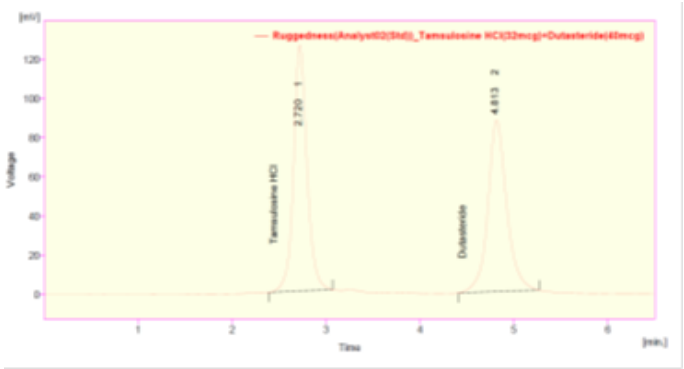

Fig.no.26: Chromatogram of Analyst 02 standard preparation

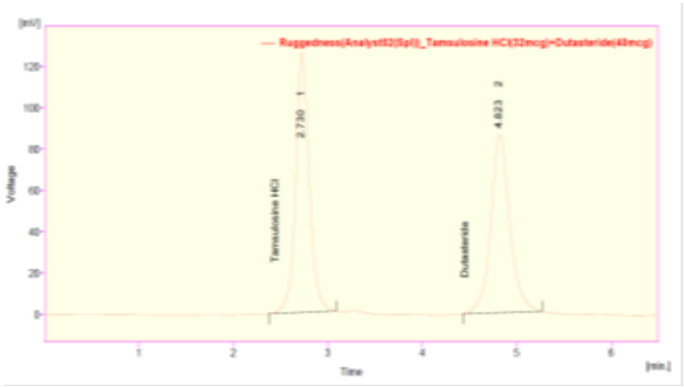

Fig.no.27: Chromatogram of Analyst 02 sample reparation

Table.No.7: Results for Ruggedness

Tamsulosin hydrochloride	%Assay	Dutasteride	%Assay
Analyst 01	99.62	**Analyst 01**	100.99
Analyst 02	99.01	**Analyst 02**	100.67
%RSD	1.09%	**%RSD**	1.14%

5. CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of Tamsulosin hydrochloride and Dutasteride was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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